

1 ***In vitro* activity of beauvericin against all developmental stages of**
2 ***Sarcoptes scabiei***

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21 **Abstract**

22 **Background:** Scabies is a frequent cutaneous infection caused by the mite *Sarcoptes scabiei* in a
23 large number of mammals including humans. As the resistance of *S. scabiei* against several
24 chemical acaricides has been previously documented, the establishment of alternative and
25 effective control molecules is required.

26 **Objectives:** In this study, the potential acaricidal activity of beauvericin was assessed against
27 different life stages of *S. scabiei* var. *suis* and, in comparison with dimpylate and ivermectin, two
28 commercially available molecules used for the treatment of *S. scabiei* infection in animals and/or
29 humans.

30 **Methods:** In our *in vitro* model, developmental stages of *S. scabiei* have been placed in Petri
31 dishes filled with Columbia agar supplemented with pig serum and different concentrations of
32 the drugs. Moreover, the toxicity of beauvericin against cultured human fibroblast skin cells was
33 evaluated using an MTT proliferation assay

34 **Results:** Beauvericin showed higher activity against adults and eggs of *S. scabiei* when
35 compared to dimpylate and ivermectin. In addition, cell sensitivity assays demonstrated low
36 toxicity of beauvericin against primary human fibroblast skin cells.

37 **Conclusion:** These results revealed that the use of beauvericin is promising and might be
38 considered for the treatment of *S. scabiei* infection.

39 **Keywords:** *Sarcoptes scabiei*, scabies, beauvericin, mycotoxin, treatment

40 **Introduction**

41 Scabies is a frequent cutaneous infection caused by the mite *Sarcoptes scabiei* in a large number
42 of mammals including human.¹ Human scabies was recently recognized as a neglected tropical
43 disease by the WHO, due to its high global prevalence estimated to be around 100-200 million
44 cases a year,² and high morbidity.³ Although primary infection with *S. scabiei* is limited to
45 severe itching and allergic rash, secondary infections with bacteria such as group A
46 streptococcus or *Staphylococcus aureus* could lead to severe acute infectious complications and
47 even death,⁴ hence the importance of early and efficient eradication of the mites.⁵ There is a
48 limited number of drugs that could be used for the treatment of scabies. Furthermore, the
49 resistance to some of the drugs of *S. scabiei* is emerging, caused by re-infection or incorrect use
50 of acaricides, may lead to an excessive and random use of treatments posing threat to patient
51 health.⁶ Less susceptible populations of *S. scabiei* may emerge from the repeated exposure to a
52 single type of acaricide. The resistance development may involve a mutation to the target site of
53 the acaricide molecule or an up-regulation for genes encoding for detoxification enzymes (Van
54 Leewan *et al.*, 2010). Therefore, the development of new acaricides with new mode of action
55 against *S. scabiei* is required.

56 Many secondary metabolites produced by fungi have been used in medicine and agriculture.⁷
57 The entomopathogenic fungus *Beauveria bassiana* is known to produce beauvericin, a secondary
58 metabolite belonging to the enniatin antibiotic family.⁸ This cyclic hexadepsipeptide was proven
59 to have many biological effects including insecticidal, antitumor, antibacterial, and antifungal
60 activity.⁹ Its mechanism of action is thought to be ionophore-induced apoptosis and DNA
61 fragmentation.⁷ Recently, there is an ongoing interest for cyclic depsipeptide as topically applied
62 medicines, treating per example, psoriasis, eczema and skin cancer (Cruz *et al.*, 2009).
63 Accordingly, beauvericin could be considered as a potential new acaricide for the treatment of

64 human and animal scabies. The objective of this study was to evaluate the *in vitro* efficacy of
65 beauvericin against the different life stages of *S. scabiei*. In addition, this study evaluated the
66 toxicity of beauvericin against cultured human fibroblast skin cells.

67

68 **Materials and method**

69 *Ethics*

70 All animals were maintained in strict accordance with good animal practices as defined by the
71 French and European code of practice for the care and use of animals for scientific purposes
72 (approval No. 02515.01). The biopsies were obtained after a written consent form was secured
73 from each individual according to an approved protocol by the Institution Review Board (IRB) at
74 the American University of Beirut (Protocol Number: DER.MK.01). The experiments were
75 conducted in accordance with Good Clinical Practice and the ethical principles of the Helsinki
76 Declaration

77 *Sarcoptes mites*

78 *Sarcoptes scabiei* mites were collected from pigs maintained at CRBM (Centre de Recherche
79 Bio Médicale), Maisons-Alfort, France. Pigs were experimentally-infected as described by
80 Mounsey.¹⁰ Inoculation was done by directly introducing mite-infected skin crusts deep into the
81 ear canals of five-week-old female piglets. Glucocorticoid treatment was initiated in naive
82 piglets one week prior to inoculation and continued. For the present study, mites were collected
83 from the pigs in weeks 15 and 16. Crusts in the external ear canal were gently removed and
84 collected in a sterile Petri dish in the morning of the *in vitro* experiments. Mites crawled out of
85 the crusts in about half an hour. Then they were picked one by one with a needle and under a
86 dissecting stereomicroscope (Nikon©, SMZ645, Lisses, France).

87 *Molecules to be evaluated*

88 Beauvericin 97% was purchased from Sigma-Aldrich. Dimpylate (diazinon) was purchased from
89 Huvepharma™ (Segre en Anjou, France) (Dimpygale®, solution 100 mg/ml). Ivermectin was
90 purchased from Boehringer-Ingelheim™ (Lyon, France) (Ivomec®, injectable solution
91 10mg/ml).

92 ***In vitro efficacy tests on mites and eggs***

93 To assess the efficacy of drugs (including beauvericin) against *S. scabiei* motile stages
94 (larvae/nymphs and females), Petri dishes filled with Columbia agar supplemented with pig
95 serum have been used for bioassays. To prepare the medium, 42g of Columbia agar (Bio-Rad,
96 Marne-la-Coquette, France) were dissolved in 1L of distilled water. The solution was autoclaved
97 for 15 min at 121°C then cooled down in a water bath at 53°C. Blood samples were obtained
98 from pigs maintained in CRBM. Tubes of blood were centrifuged at 4500 rpm for 10 min at 4°C.
99 The resulting supernatant was designated serum. For the preparation of each Petri dish, one ml of
100 serum was added to 18 ml of Columbia agar medium at 53°C. Drugs to be tested were
101 incorporated into the medium following the method described by Brimer^{11,12} with slight
102 modifications. The mycotoxin beauvericin and two acaricide drugs (dimpylate and ivermectin)
103 were tested with different concentrations. The absence of drug concentration was considered as
104 negative control. The required volumes of serum-supplemented Columbia agar and molecules
105 were pipetted in a tube and quickly transferred to a 9 cm sterile plastic Petri dish under a flow
106 cabinet. Petri dishes were kept under the flow cabinet until the agar is solidified, and stored
107 upside down at 4°C until use. The efficacy of 3 different concentrations 0.5, 5, and 50 µM was
108 evaluated. Five females and five nymphs or larvae were inoculated in the middle of the plates
109 and examined at 1, 2, 3, 4, 5, 6, 7, 8 and 24h after inoculation for survival assessment of motile
110 stages at room temperature. Mites were considered dead when no movement occurred under the
111 microscope during 5 min even after a gentle stimulation with a dissecting needle. After each
112 inspection, mites were moved again to the center of the plate using a dissecting needle to lower
113 chances of runaways.

114 To assess the efficacy of chemical products against *S. scabiei* eggs, 10 eggs were manually
115 isolated using a fine needle and placed in the middle of beauvericin, dimpylate, or ivermectin-

116 supplemented agar plates under the dissecting stereomicroscope as described above. Petri dishes
117 were maintained at 37°C in an incubator for 5 days to promote egg development. Newly hatched
118 larvae were recorded and removed from the Petri dishes.

119 Five replications were performed in three biological replicates, making it a total of 150 motile
120 stages and 150 eggs observed for each treatment.

121 ***Primary fibroblast culture***

122 Human skin biopsies from healthy volunteer patients were delivered to the laboratory in culture
123 medium (RPMI 1640: 450 ml, FBS: 50 ml, and penicillin-streptomycin solution x100: 1 ml).
124 Each biopsy was transferred into a sterile Petri dish and rinsed with PBS to eliminate blood and
125 debris. Two ml collagenase (Worthington™) were added to the medium before mincing the
126 tissue with a scalpel. After incubation at 37°C for 1h, the digested tissue was transferred to a
127 15 ml conical tube and the Petri dish was rinsed twice with 2 ml of the medium and the liquid
128 was collected in the same tube and span down at 200g for 5 min at room temperature. The pellet
129 was washed twice with 3 ml of the medium to remove the collagenase, resuspended with 5 ml of
130 the same medium and transferred to T25 flask. Finally, the cells were cultured in a 37°C
131 humidified air incubator with 5% CO₂. When there were sufficient cells, the latter were detached
132 with trypsin and plated in another dish for further proliferation.

133 ***Beauvericin cytotoxicity assessment***

134 The cell death rates of treated fibroblast were used as an indicator to assess the cytotoxicity of
135 beauvericin. Cultured cells were transferred to 96 wells plate and treated with 12 different
136 concentrations of beauvericin in triplicates (0-50 µM) when they reached 50-60% confluence.
137 Cells were continuously exposed to the drugs for 48h and subsequently assessed for cell death.
138 The viability of the cells was assessed based on their metabolic activity using the MTT
139 proliferation assay,¹³ as follows: 24h pre-treatment, fibroblast cells were starved in 100µl FBS

140 free media. After starvation, fibroblast cells were exposed to the drugs as described above. Four
141 hours prior to the end of the treatment, 10 μ l MTT dye (100 mg Thiazolyl Blue Tetrazolium
142 Bromide, Sigma Aldrich, 20 ml PBS) were added to the wells. In addition, 100 μ l MTT stop
143 solution (12mM HCl, 0.05% isobutanol, 10% SDS) was added to cells before incubation at 37°C
144 overnight. After 24h, the absorbance was measured at 550 nm on an ELISA plate reader. All
145 tests were carried out in triplicates of three biological replicates.

146 *Statistical analyses*

147 Efficacy data of all treatments against *S. scabiei* were analyzed by Kaplan Meier survival curves
148 using software Statistical Package for the Social Sciences (SPSS, version 25).¹⁴ The statistical
149 differences between data obtained with each treatment and the control for each experiment were
150 measured by Log-rank test expressed by Chi-2 results and P-values (degree of freedom (df) = 1).
151 P-value of ≤ 0.05 was considered significant. The lethal concentration (LC₅₀) and lethal time
152 (LT₅₀) necessary to kill half of the mite's population in addition to the lethal concentration
153 (LC₅₀) necessary to kill half of fibroblast cells and their standard error were calculated using the
154 probit regression analysis in (SPSS). The median time of 50% hatching (HT₅₀) of the eggs was
155 assessed.

156 LC₅₀ of all treatments were analyzed by the statistical comparison test of means (ANOVA)
157 using SPSS. The Tukey test was used at the 5% threshold for the separation of means.

158 **Results**

159 The three molecules, beauvericin, dimpylate, and ivermectin were highly efficient against motile
160 stages of *S. scabiei* mites (Table 1 and Figure 1). The mortality rates in the control group were
161 below 5% during the first 8h post-exposure. The survival and hatching curves of mites and eggs
162 exposed to different drugs are presented in Figure 1. In all tests, significant differences were
163 found between each molecule and the control except for the tests against *S. scabiei* eggs with 0.5

164 μM of dimpylate and ivermectin. The highest mortality rates of all developmental stages were
165 recorded with a concentration of 50 μM of all drugs. The efficiency of each treatment decreased
166 steadily with the decrease in the concentration of the molecules: the lowest mortality rates were
167 recorded within the plates supplemented with 0.5 μM of all drugs. Overall, a differential effect
168 between the molecules and the concentration being used was notable 1h post-exposure (Table 1).

169 The efficacy of the molecules at different concentrations can be put into the following order
170 based on their chi-square value: beauvericin > ivermectin > dimpylate and ivermectin >
171 dimpylate > beauvericin against females and immature forms, respectively (Table 1). The
172 survival capacity seemed to be different according to the developmental stage of the mites.
173 Dimpylate and ivermectin had higher efficacy on *S. scabiei* immature motile stages when
174 compared to females; whereas, beauvericin displayed a higher efficacy on females at all
175 concentrations (Table 1).

176 The activity of all three molecules against *S. scabiei* eggs was evaluated for 5 days. Results
177 obtained with beauvericin, dimpylate or ivermectin were significantly different from those in the
178 control group (Table 1). Among all the molecules tested against the eggs of *S. scabiei*,
179 beauvericin demonstrated the best inhibition of hatching effect, when testing a concentration of
180 50 μM . The second-highest activity was recorded when treating eggs with the same
181 concentration of dimpylate. The lowest activity was recorded at a concentration of 0.5 μM of
182 dimpylate or ivermectin with no significant statistical difference compared to the negative
183 control (Table 1).

184 LT_{50} values were different between treatment groups against the motile stages of *S. scabiei*
185 (Table 1). The highest LT_{50} values (5.6 and 4.7h) were observed with a concentration of 0.5 μM
186 of beauvericin and dimpylate against females and nymphs/larvae, respectively. The lowest LT_{50}
187 values (1.1 and 1 h) were observed with a concentration of 50 μM of dimpylate and ivermectin

188 against females and nymphs/larvae, respectively. The median time for hatching of 50% of the
189 eggs was also recorded in this study (Table 1).

190 A significant difference was recorded between LC_{50} values of the drugs at 1h ($F = 68.779$, $df = 2$,
191 $P < 0.05$), 2h ($F = 12.809$, $df = 2$, $P < 0.05$), 4h ($F = 145.902$, $df = 2$, $P < 0.05$), 5h ($F = 59.758$, df
192 $= 2$, $P < 0.05$), and 6h- post exposure against females ($F = 12.809$, $df = 2$, $P < 0.05$). The effect of
193 the drugs was not notable 3h post exposure against females ($F = 0.39$, $df = 2$, $P > 0.05$). A
194 significant difference was also notable between LC_{50} values of molecules at any given time of
195 the test against larvae: 1h ($F = 367.273$, $df = 2$, $P < 0.05$), 2h ($F = 1423.942$, $df = 2$, $P < 0.05$), 3h
196 ($F = 108.356$, $df = 2$, $P < 0.05$), 4h ($F = 86.263$, $df = 2$, $P < 0.05$), 5h ($F = 94.776$, $df = 2$, $P < 0.05$),
197 and 6h post exposure ($F = 68.387$, $df = 2$, $P < 0.05$). Median times of 50% hatching of the eggs
198 are presented in Table 1. The highest median time for hatching (2.9 days) was recorded with a
199 concentration of 50 μM of beauvericin. A significant difference was recorded between LC_{50}
200 values of the molecules against eggs at 5 days post-exposure ($F = 42.709$, $df = 2$, $P < 0.05$).

201 The cytotoxic effect of beauvericin was assessed at 48h post-exposure. The mycotoxin caused a
202 dose dependent reduction in cell viability and lethal concentration 50 was calculated. The human
203 fibroblast cells were moderately sensitive to beauvericin toxicity and LC_{50} was 4.8 μM .

204 **Discussion**

205 The mortality rate in the control groups was low (< 5%) during the first 8h of the test indicating
206 suitable conditions of the bio-assays. The survival of *S. scabiei* outside its host is considered to
207 be the first limitation of *in vivo* studies. After 24h, the “natural” mortality rate at room
208 temperature reached 19% which indicates that mortalities recorded at 24h may not be caused
209 only by the acaricide effect of the tested molecules; therefore, results recorded at 24h post
210 inoculation are discarded.

211 The efficacies of macrocyclic lactones (including ivermectin) or organophosphates (like
212 dimpylate) against *S. scabiei* have been previously evaluated.^{12,15,19,22} However, most studies
213 evaluated the efficacy of such molecules against motile stages of *S. scabiei* with no
214 differentiation of the life stage. Studies evaluating the efficacy of acaricides against specific life
215 stages of *S. scabiei* are few. The present study demonstrated that the application of dimpylate
216 and ivermectin caused higher survival rates among females compared to immature forms. These
217 results are in accordance with those from Mounsey¹⁷ who observed that *S. scabiei* nymphs and
218 larvae were more vulnerable than females to ivermectin and moxidectin. Generally, larvae are
219 more sensitive due to their greater surface area/volume ratio meaning more drug is absorbed
220 through the cuticle during *in vitro* exposure. On the contrary, in the present study, *S. scabiei*
221 females seemed to be more vulnerable to beauvericin than immature forms. These results are in
222 accordance with those from Fu-Xing *et al.*, 2002 reporting a higher induction of two kinds of
223 insecticides metabolic detoxifying enzymes by larvae of *Musca domestica* when compared to
224 adults.

225 The resistance of *S. scabiei* to commercial products exists and might increase in the future;
226 whereas, treatment failures of scabies infections in animals and humans have been previously
227 reported.²⁰⁻²³ Beauvericin is known to be an ionophoric cyclodepsipeptide which forms

228 complexes with cations and increases the permeability of biological membranes.²⁴⁻²⁶ Given the
229 non-similarity of its mode of action to that of the commonly used neuro inhibitors, a cross-
230 resistance of *S. scabiei* mites against beauvericin is unlikely to happen.

231 The three drugs selected for the bioassays had a low LT_{50} value, indicating a rapid effect on the
232 mites. For beauvericin and dimpylate, LT_{50} values were related to the concentration of the
233 molecules (death occurred more rapidly among mites treated with higher concentrations).

234 The dose-response test recorded low LC_{50} values indicating high efficacy of all drugs at killing *S.*
235 *scabiei* mites. Moreover, LC_{50} values obtained in the present study were low when compared to
236 those obtained by Mounsey.¹⁷ The latter study reported that 50.5 μM of ivermectin is required to
237 kill 50% of *S. scabiei* mites 1h post-exposure (versus 45.1 μM in our study). This result could be
238 explained by the fact that the strain of *S. scabiei* and/or the assay conditions used in the present
239 study were different.

240 The next generation of scabicide molecules needs to target *S. scabiei* eggs and ensuing
241 developmental stages. In the present study, high hatching rates were recorded among the eggs in
242 contact with dimpylate and ivermectin. These results are in accordance with Dourmishev²⁷, Usha
243 and Nair²⁸ demonstrating that presently used acaricides have a very limited inhibition of hatching
244 activity. The present study demonstrated that beauvericin was moderately active against the eggs
245 of *S. scabiei*.

246 Data about beauvericin cytotoxicity, especially against skin cell line are lacking. This lack of
247 data could explain why there are no maximum guidance levels. The present study assessed for
248 the first time the cytotoxic activity of beauvericin against primary fibroblast human skin cells.
249 Heilos *et al.*³⁰ investigated the cytotoxic activity of beauvericin against the principal constituent
250 of the epidermis, HEK2 keratinocyte ($IC_{50} = 5.4 \mu\text{M}$). Furthermore, systemic kinetics and effect
251 of beauvericin should also be assessed since the cyclic depsipeptide is transdermally absorbed³¹.

252 The impact of beauvericin was also assessed against several nucleated human cells (IC50s
253 included human intestinal cell line Caco 2= 3.9 μ M; human liver cell line HEPG2 liver= 3.4 μ M;
254 human normal vascular endothelial cells HUVEC = 2.4 μ M)³⁰. The latter reviews revealed
255 cytotoxic activity against human cell lines at relatively low concentrations; nevertheless, the
256 present study demonstrated that the therapeutic index of beauvericin for scabies infection could
257 be high. The susceptible dose against motile stages of *S. scabiei* are extremely low when
258 compared that of all human cell lines. Furthermore, a study conducted by Taevernier *et al.*³¹
259 demonstrated that beauvericin concentration was 21 times higher in the epidermis than in the
260 dermis after topical application of the mycotoxin. Moreover, transdermal kinetics is mediated by
261 the outermost layer of the skin providing a protective reservoir for cyclic depsipeptides.³¹
262 In conclusion, this study presented the first evidence of *in vitro* efficacy of the mycotoxin
263 beauvericin against different developmental stages of *S. scabiei* mites. Beauvericin showed
264 higher efficacy against females and eggs of *S. scabiei* when compared to the two commercially
265 available acaricides, dimpylate, and ivermectin. Furthermore, beauvericin had low cytotoxicity
266 against fibroblasts. These preliminary results indicated that beauvericin may be considered as a
267 new scabicide molecule. Further studies assessing the possibility of beauvericin application to
268 treat scabies in humans or sarcoptic mange in animals are required. Bioavailability, toxicokinetic
269 properties, distribution, absorption, metabolization and excretion of the mycotoxin need now to
270 be documented. Studies measuring and confirming that the maximum concentration of
271 beauvericin in the skin of patients are within the *in vitro* susceptibility range are crucial.
272

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279 **Transparency declarations (conflicts of interest)**

280 None to declare

281 **REFERENCES**

- 282 **1.** Walton SF, Currie BJ. Problems in diagnosing scabies, a global disease in human and animal
283 populations. *Clin Microbiol Rev.* 2007; **20**: 268-79.
- 284 **2.** Karimkhani C, Colombara DV, Drucker AM et al. The global burden of scabies: A cross-
285 sectional analysis from the global burden of disease study 2015. *Lancet Infect*
286 *Dis.* 2017; **17**: 1247-54.
- 287 **3.** Engelman D, Cantey PT, Marks M et al. The public health control of scabies: Priorities for
288 research and action. *The Lancet.* 2019; **394**: 81-92
- 289 **4.** Lynar S, Currie BJ, Baird R. Scabies and mortality. *Lancet Infect Dis.* 2017; **17**: 1234.
- 290 **5.** McCarthy JS, Kemp DJ, Walton SF et al. Scabies: More than just an irritation. *Postgrad Med*
291 *J.* 2004; **80**: 382-7
- 292 **6.** Walton SF, Myerscough MR, Currie BJ. Studies in vitro on the relative efficacy of current
293 acaricides for *Sarcoptes scabiei* var. *hominis*. *Trans R Soc Trop Med Hyg.* 2000; **94**: 92-6.
- 294 **7.** Wang Q, Xu L. Beauvericin, a bioactive compound produced by fungi: A short
295 review. *Molecules.* 2012; **17**: 2367-77.
- 296 **8.** Vega FE, Meyling NV, Luangsa-ard JJ et al. Fungal entomopathogens. *Insect*
297 *Pathol.* 2012; **2**: 171-220.
- 298 **9.** Mallebrera B, Prosperini A, Font G et al. In vitro mechanisms of beauvericin toxicity: A
299 review. *Food Chem Toxicol.* 2018; **111**: 537-45.
- 300 **10.** Mounsey K, Ho M, Kelly A et al. A tractable experimental model for study of human and
301 animal scabies. *PLoS Negl Trop Dis.* 2010; **4**: e756.

- 302 **11.** Brimer L, Henriksen SA, Gyrd-Hansen N et al. Evaluation of an in vitro method for acaricidal
303 effect. activity of parathion, phosmet and phoxim against *Sarcoptes scabiei*. *Vet*
304 *Parasitol.* 1993; **51**: 123-35.
- 305 **12.** Brimer L, Bønløkke L, Pontoppidan C et al. A method for in vitro determination of the
306 acaricidal effect of ivermectin using *Sarcoptes scabiei* var. *suis* as test organism. *Vet*
307 *Parasitol.* 1995; **59**: 249-55.
- 308 **13.** Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to
309 proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; **65**: 55-63.
- 310 **14.** IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY:
311 IBM Corp.
- 312 **15.** Huffam SE, Currie BJ. Ivermectin for *Sarcoptes scabiei* hyperinfestation. *Int J Infect*
313 *Dis.* 1998; **2**: 152-4.
- 314 **16.** Glaziou P, Cartel JL, Alzieu P et al. Comparison of ivermectin and benzyl benzoate for
315 treatment of scabies. *Trop Med Parasitol.* 1993; **44**: 331-2.
- 316 **17.** Mounsey KE, Walton SF, Innes A et al. In vitro efficacy of moxidectin versus ivermectin
317 against *Sarcoptes scabiei*. *Antimicrob Agents Chemother.* 2017; **61**: 381.
- 318 **18.** Baishya SK, Das A, Bardoloi RK. Therapeutic efficacy of dormectin, diazinon and
319 deltamethrin against mange mite infestation in pig. *Indian J Hill Farming (India).* 2003; **16**:
320 82-5
- 321 **19.** Feyera T, Admasu P, Abdilahi Z et al. Epidemiological and therapeutic studies of camel mange
322 in Fafan zone, eastern Ethiopia. *Parasite Vector.* 2015; **8**: 612.

- 323 **20.** Aussy A, Houivet E, Hébert V et al. Risk factors for treatment failure in scabies: A cohort
324 study. *Br J Dermatol.* 2019; **180**: 888-93.
- 325 **21.** Mounsey KE, Holt DC, McCarthy JS et al. Longitudinal evidence of increasing in vitro
326 tolerance of scabies mites to ivermectin in scabies-endemic communities. *Arch*
327 *Dermatol.* 2009; **145**: 840-1.
- 328 **22.** Currie BJ, Harumal P, McKinnon M et al. First documentation of *in vivo* and *in vitro*
329 ivermectin resistance in *Sarcoptes scabiei*. *Clin Infect Dis.* 2004; **39**: e-e12.
- 330 **23.** Terada Y, Murayama N, Ikemura H et al. *Sarcoptes scabiei* var. *canis* refractory to ivermectin
331 treatment in two dogs. *Vet Dermatol.* 2010; **21**: 608-12.
- 332 **24.** Toman P, Makrlík E, Vaňura P. On the complexation of the sodium cation with beauvericin:
333 Experimental and theoretical study. *Monatsh Chem.* 2011; **142**: 779-82.
- 334 **25.** Wätjen W, Debbab A, Hohlfeld A et al. The mycotoxin beauvericin induces apoptotic cell
335 death in H4IIE hepatoma cells accompanied by an inhibition of NF- κ B-activity and modulation
336 of MAP-kinases. *Toxicol Lett.* 2014; **231**: 9-16.
- 337 **26.** Lu C, Lin H, Chen B et al. Beauvericin-induced cell apoptosis through the mitogen-activated
338 protein kinase pathway in human nonsmall cell lung cancer A549 cells. *J Toxicol*
339 *Sci.* 2016; **41**: 429-37.
- 340 **27.** Dourmishev AL, Dourmishev LA, Schwartz RA. Ivermectin: Pharmacology and application in
341 dermatology. *Int J Dermatol.* 2005; **44**: 981-8.
- 342 **28.** Usha V, Nair TG. A comparative study of oral ivermectin and topical permethrin cream in the
343 treatment of scabies. *J Am Acad Dermatol.* 2000; **42**: 236-40.

- 344 **29.** Jestoi M. Emerging fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and
345 moniliformin—A review. *Crit Rev Food Sci Nutr.* 2008; **48**: 21-49.
- 346 **30.** Heilos D, Rodríguez-Carrasco Y, Englinger B et al. The natural fungal metabolite beauvericin
347 exerts anticancer activity *in vivo*: A pre-clinical pilot study. *Toxins.* 2017; **9**: 258.
- 348 **31.** Taevernier L, Veryser L, Roche N, Peremans K, Burvenich C, Delesalle C, De Spiegeleer B.
349 Human skin permeation of emerging mycotoxins (beauvericin and enniatins). *J. Expo. Sci.*
350 *Environ. Epidemiol.* 2016; **26**, 277–287.

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352

353 **Legend of figure**

354 **Figure 1:** Curves representing survival (of motile stages) and hatching (of eggs) of *S. scabiei*

355 exposed to acaricide molecules at different concentrations: a) 0.5 μM , b) 5 μM , and c) 50 μM .

356 For each treatment, observed survivals and hatchings are presented using curves with markers;

357 filled circle: beauvericin; filled square: dimpylate; filled triangle: ivermectin; open circle: control

358

359

1 **Table 1:** Lethal time (LT₅₀) to kill *Sarcoptes scabiei* females, nymphs and larvae or eggs and
 2 statistical differences between data obtained with each drug (beauvericin, dimpylate, and
 3 ivermectin).
 4

Drugs and concentrations		Females			Nymphs & larvae			Eggs		
		X ²	P	LT ₅₀ ± S.E. (h)	X ²	P	LT ₅₀ ± S.E. (h)	X ²	P	HT ₅₀ ± S.E. (h)
Beauvericin	0.5 μM	112	<0.05	3.4±0.1	34.4	<0.05	4.7±0.2	39	<0.05	2.3±0.1
	5 μM	159.7	<0.05	1.9±0	79	<0.05	4.1±0.2	54.2	<0.05	2.5±0.1
	50 μM	165.9	<0.05	1.4±0	124	<0.05	2.3±0.1	85.9	<0.05	2.9±0.1
Dimpylate	0.5 μM	5.9	<0.05	5.6±0.17	39.7	<0.05	4.5±0.2	1.3	>0.05	1.5±0
	5 μM	154.3	<0.05	3.2±0.1	172	<0.05	2±0.1	12.1	<0.05	1.8±0
	50 μM	167	<0.05	1.1±0	167.3	<0.05	1.1±0	38.8	<0.05	2.3±0.1
Ivermectin	0.5 μM	151.4	<0.05	2.8±0.1	166.5	<0.05	1.6±0	0.3	>0.05	1.4±0
	5 μM	156.8	<0.05	2.4±0.1	166.9	<0.05	1.2±0	6.5	<0.05	1.6±0
	50 μM	166.5	<0.05	1.6±0.1	164.3	<0.05	1±0	19.8	<0.05	1.9±0.1

5
 6 Note: X²: Chi-square value; LT₅₀: Lethal Time to kill 50% of mites; S.E.: Standard error; h:
 7 Hour; P: probability value; μM: micromolar.

1
 2 **Table 2:** Concentrations of different molecules required to kill 50% of *S. scabiei* mites (females,
 3 nymphs/larvae and eggs).

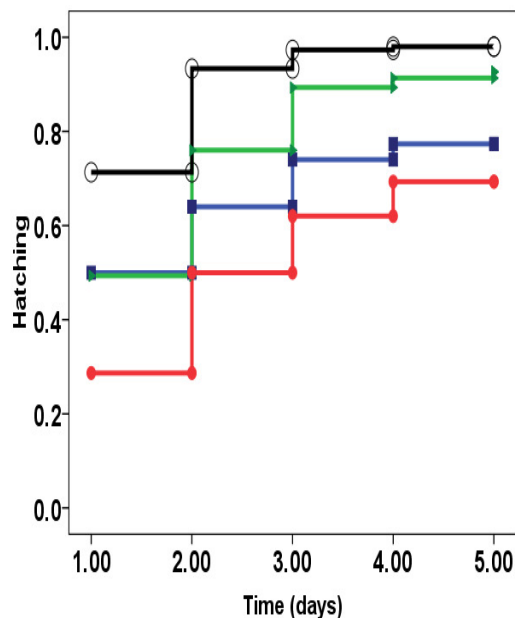
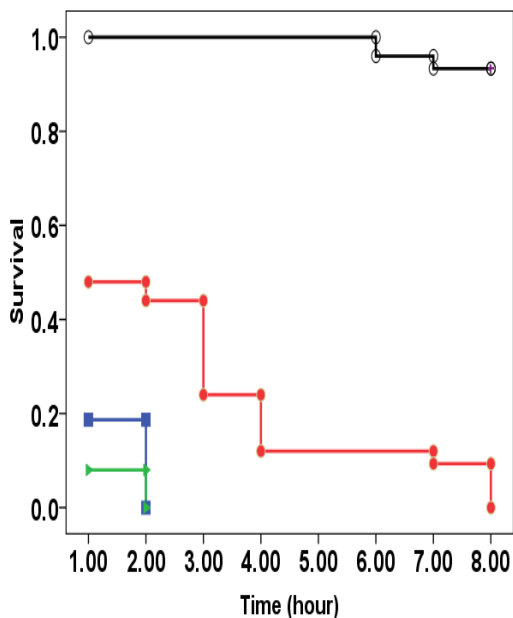
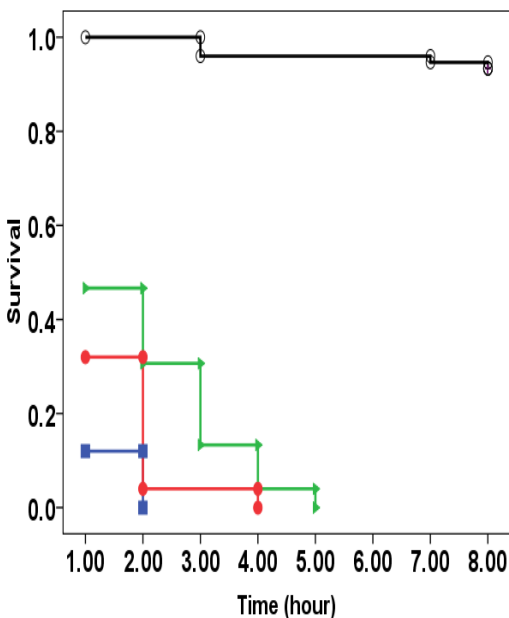
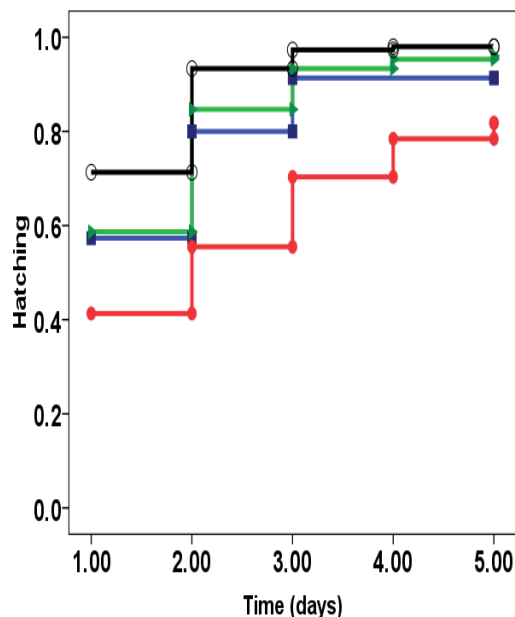
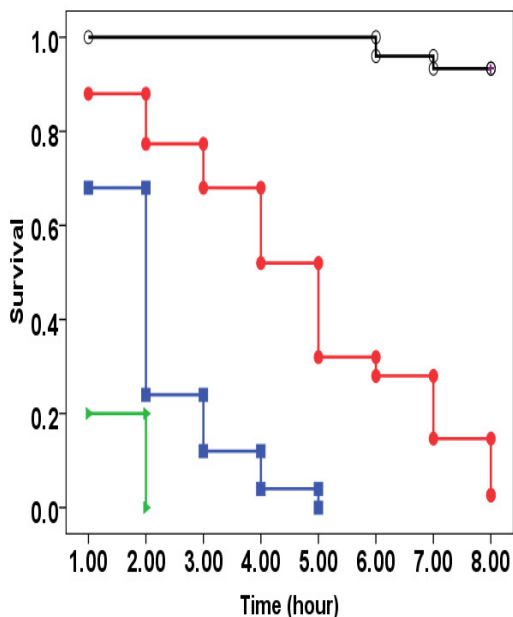
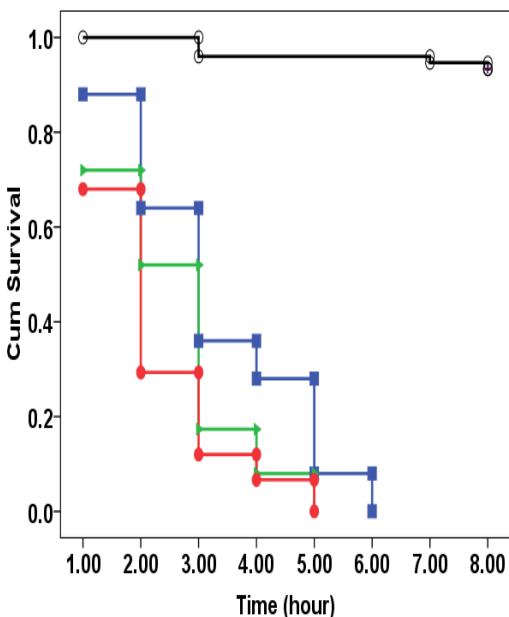
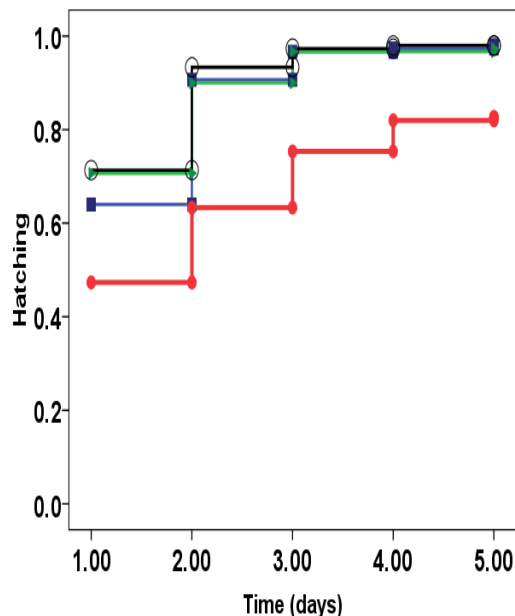
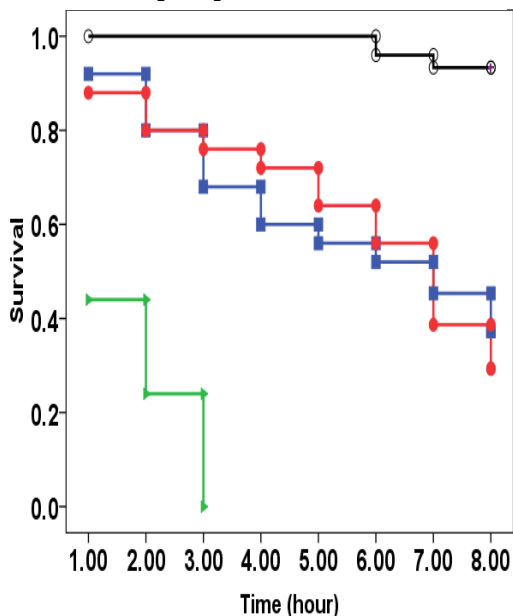
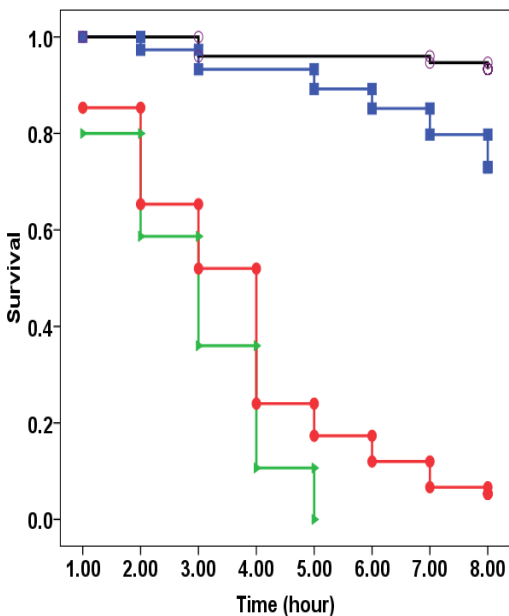
		LC ₅₀ (µM) ± S.E.															
Exposure	1h		2h		3h		4h		5h		6h		7h		8h		5 days
	Females	Nymphs & larvae	Females	Nymphs & larvae	Females	Nymphs & larvae	Females	Nymphs & larvae	Females	Nymphs & larvae	Females	Nymphs & larvae	Females	Nymphs & larvae	Females	Nymphs & larvae	Eggs
Beauvericin	34.7± 3.9 ^c	48.1 ±1.7 ^b	7.8± 5.1 ^a	39.5± 3 ^b	2±0.9 _a	28.4± 2.6 ^b	0.7±0 .2 ^b	18.9± 1.9 ^b	0.3±0 _b	13.7± 1.3 ^b	0.3±0 _b	10.4± 1.2 ^b	0.3±0 _a	1.7±0. 6 ^b	0.3±0 ^a	0.9±0 ^a	77.5 ±1.8 ^a
Dimpylate	7.09± 0 ^a	6.9± 1 ^a	5.9± 0.2 ^a	3.3±0 .2 ^a	4.1±0 .1 ^a	2.3±0 .7 ^a	3.9±0 .1 ^a	1.7±0 _a	2.5±0 .2 ^a	0.5±0 _a	1.5±0 .1 ^a	0.5±0 _a	1.1±0 .4 ^a	0.4±0 _a	0.8±0. 1 ^b	0.4±0 ^a	77.5 ±1.8 ^a
Ivermectin	45.1± 0.9 ^b	5.7± 0.8 ^a	26±1 .5 ^b	0.4±0 _a	4.7±3 .9 ^a	0.3±0 _a	0.3±0 _b	0.3±0 _a	0.3±0 _b	0.3±0 _a	0.3±0 _b	0.3±0 _a	0.3±0 _a	0.3±0 _a	0.3±0 ^a	0.3±0 ^a	202.6 ±16.5 ^b

4
 5 Note: *LC*₅₀: Lethal Concentration to kill 50% of mites; *S.E.*: Standard error; *h*: Hour; µM:
 6 micromolar
 7 *Values followed by the same letter in the same column are not significantly different at
 8 the 5% threshold

Females

Nymphs & larvae

Eggs



● beauvericin

■ dimpylate

▸ ivermectin

○ Control